Use of γ irradiation to eliminate DNA contamination for PCR

Jean-Marc Deragon, Daniel Sinnett, Grant Mitchell, Michel Potier and Damian Labuda
Service de Génétique Médical, Hôpital Ste-Justine, Université de Montréal, 3175 Chemin Sainte-Catherine, Montréal, Québec H3T 1C5, Canada

Submitted September 10, 1990

A major problem encountered in the routine use of the polymerase chain reaction (PCR) is contamination of the reaction mixture with minute amounts of extraneous DNA which can serve as a PCR template and lead to confusion in interpreting results. Ultraviolet irradiation or DNaseI treatment of samples prior to PCR have recently been proposed to eliminate such DNA contamination (1, 2). We tested whether γ radiation, which can be accurately quantitated and penetrates water efficiently could suppress the amplification of small amounts of genomic and plasmid DNA.

We first used genomic DNA as a template to amplify a 294 bp fragment from the 3′ region of the human L1 sequence. Since L1 sequences are present in more than 10^5, copies in human cells, this provides a sensitive measure of human genomic DNA contamination. A dose of 150 krad eliminated amplification from 0.1 ng of genomic DNA without adversely affecting the PCR reagents (figure, lane 6). When we used plasmid templates to amplify fragments of 280 and 717 bp, a radiation dose of 200 krad eliminated amplification at a plasmid copy number of 10^4 (figure, lanes 10 and 16). After irradiation, the PCR mixture remained capable of amplifying freshly-added target DNA at doses of up to 400 krad although reduced efficiency was seen at higher doses. As shown in lanes 11 and 12 (figure) amplification was detectable after irradiation in the presence of $>10^5$ copies of plasmid DNA. However, in the presence of 0.1 ng of genomic DNA (the equivalent of 15 cells) or of 0.1 pg of plasmid 200 krad of γ radiation was sufficient to eliminate detectable contamination in the three target systems studied, even after 40 amplification cycles.

The inactivation of DNA templates by γ irradiation probably occurs by reaction with free radicals formed from the ionisation of water. The efficiency of this inactivation undoubtedly depends upon several factors including the length of the amplified DNA segment and the precise composition of the PCR mixture. Therefore, irradiation conditions will have to be established for each amplification system. Gamma-irradiation provides one option for the suppression of PCR amplification from trace amounts of contaminating DNA. Since γ irradiation is increasingly used in research and in medical situations such as the treatment of cancer and the preparation of blood products, γ sources are available in many centres.

REFERENCES

1. Sarkar,G. and Sommer,S.S. (1990) *Nature* **343**, 27.
2. Furrer,B., Candrian,U., Wieland,P. and Luthy,J. (1990) *Nature* **346**, 324.
3. Gibbs,P.E.M., Zielinski,R., Boyd,C., and Dugaiczyk,A. (1987) *Biochemistry* **26**, 1332–1343.
4. Mitchell,G.A., Looney,J.E., Brody,L.C., Steel,G., Suchanek,M., Engelhardt,F.J., Willard,H.F. and Valle,D. (1988) *J. Biol. Chem.* **238**, 14288–14295.

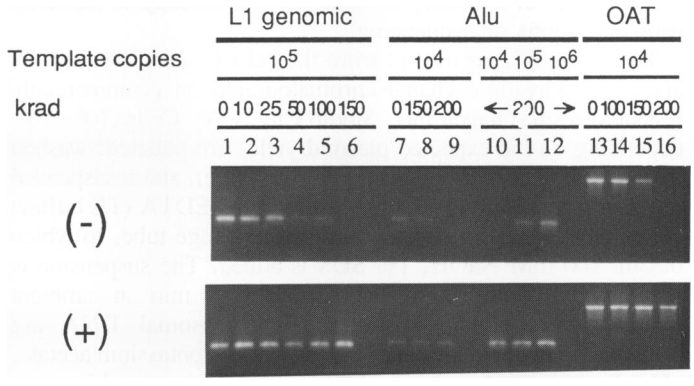

Figure 1: Amplification products of gamma-irradiated DNA. The target DNAs, the length of the amplified fragment and the radiation doses are indicated above each lane. A 100 μl solution containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2.0 mM $MgCl_2$, 0.01% gelatin, 200 μmol/L each of dATP, dCTP, dGTP and dTTP, 1 μmol/L of each primer and the indicated amounts of DNA were irradiated in 500 μl Eppendorf tubes using a Gammacell model 220 irradiator (Atomic Energy of Canada Limited) at a dose rate of about 1.2 Mrad/h. After irradiation, 50 μl aliquots were obtained from the reaction mixture, and to one (+) we added nonirradiated genomic or plasmid DNA, as indicated. 1.25 units of Taq polymerase (BRL) were added to each reaction mixture. 40 cycles of amplification were performed as follows: 30 sec 94°C, 45 sec 60°C and 45 sec 72°C, followed by 5 min at 72°C. 10 μl of each reaction mixture were electrophoresed directly on a 1.4% agarose gel and stained with ethidium bromide. The oligonucleotides used were: L1–5′:TGTGGAAGTCAGTGTGGCGA, L1–3′: CCAATTTCATCCATGTCCCT, Alu-5′: TAATACGACTCACTATAGGGCCGGGCGCGGTGGCTCA, Alu-3′: CTCGCTCTGAGGCAGAGTT, OAT-5′: CTTGATCACCAGCGGAGGC, OAT-3′: TGCTATCTCCAGTTCCAG. The Alu-containing plasmid was pHAFP5.5 (3) provided by Achilles Dugaiczyk and the ornithine-δ-aminotransferase (OAT) plasmid (4) contained a human liver cDNA insert provided by David Valle.